

# Fractionation and Reconstitution Techniques for Studying Water-Retention Properties of Wheat Flours<sup>1</sup>

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## ABSTRACT

Water-retention capacities (WRC) of reconstituted flours were 10 to 20% higher than those for the parent normal flours when a standard fractionation and reconstitution (F&R) procedure was used. WRC of reconstituted flours were not affected by freeze-drying all fractions as compared with air-drying, by fractionating at zero temperatures, by variations in particle-size distribution, or by omission of the water-solubles. Longer reconstitution mixing times lowered WRC slightly. A simple blend of fractions had a slightly higher WRC than the corresponding reconstituted flour. Reconstituted flours had high inherent maltose values but apparently normal damaged-starch values. As compared with flours from the standard F&R procedure, flours reconstituted from the fractions of an acetic acid extraction procedure had higher WRC, and flours reconstituted from fractions of a kneading method in which the dough was formed with sodium chloride solution had lower WRC.

The use of fractionation and reconstitution (F&R) techniques had been planned as part of a study on the water-holding properties of wheat flour. The first F&R project was to locate the flour fractions causing the differences between wheat classes in water-retention capacities (WRC) and alkaline water-retention capacities (AWRC). The AWRC test has proved to be a valuable tool in soft wheat flour evaluation. It and WRC are highly correlated with cookie diameter and absorption tests, but there is no correlation between these tests and bread loaf volume (1).

Ideally, F&R procedures should separate flour into well-defined fractions, process these fractions to stable preparations suitable for analytical tests, and recombine the fractions to reconstituted flours that have properties identical with those of the original flour (2). However, the use of reconstituted flours for the first time in a particular test may give very different results from those obtained with the parent normal flours. In preliminary experiments, reconstituted flours had higher WRC than did the parent normal flours. This study reports the effect of several variables in the F&R procedures on the WRC of reconstituted flours.

## MATERIALS AND METHODS

### Flours

Three straight-grade flours were milled from single-variety wheat composites on the Buhler mill<sup>2</sup>. Rio was a typical hard red winter (HRW) flour, Gaines was a typical soft white winter (SWW) flour, and Omar was a typical club wheat flour. These flours conformed to the varietal ratings established in this laboratory (3). Table I gives the analytical data.

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<sup>2</sup>A trademark name of the Buhler Corporation, 8925 Wayzata Blvd., Minneapolis, Minn. 55426.

TABLE I. ANALYTICAL DATA FOR FLOURS

Wheat Class	Variety	Moisture %	Protein <sup>a,b</sup> %	WRC %	AWRC %	Mass Mean Size $\mu$	Damaged Starch %
HRW	Rio	13.8	9.45	68.2	70.5	54	7.4
SWW	Gaines	12.7	9.13	52.3	53.7	45	3.8
Club	Omar	13.4	9.53	50.6	51.4	46	3.8

<sup>a</sup>All data are on a 14% m.b.

<sup>b</sup>N  $\times$  5.7.

#### Standard Flour Fractionation Method

The fractionation method used for most of this study was a kneading procedure of a mechanically mixed dough (4). Mechanical mixing was continued only until a suitable dough was formed; generally less than 1 min. was required. Kneading and other separation procedures were carried out under room conditions. The water extract was concentrated under reduced pressure and freeze-dried. Gluten, tailings, and starch were air-dried and ground. Dry matter and protein recoveries were 98 to 99%.

#### Other Flour Fractionation Methods

Three other fractionation methods were studied. Kneading of a mechanically mixed dough with a dilute sodium chloride solution used to form the dough was studied (5). Flour (300 g.) was mixed for 1 min. with 160 ml. water in which 5.25 g. sodium chloride had been dissolved. This dough was then fractionated as in the standard method; distilled water was used in all subsequent stages.

Yamazaki (6) introduced a fractionation method that avoided any mechanical mixing that might cause gluten or dough development. Essentially the same method was used in extensive bread flour studies (7). In the present study, the original method was followed exactly with 300-g. batches of flour.

Flour was also fractionated by an acetic acid extraction method that avoided dough development (8).

For all fractionation methods, the water-solubles were freeze-dried while the gluten, tailings, and starch were air-dried (an exception was the experiment comparing freeze-dried fractions with air-dried fractions). Table II gives the analytical data for all fractionations.

#### Flour Reconstitution

Water-solubles, gluten, tailings, and starch in the proportions obtained in fractionation were blended, and 50-g. lots were mixed with distilled water in a 100-g. National Manufacturing Company dough mixer to doughs of optimum consistency. The doughs were freeze-dried and ground on a Hobart Manufacturing Company coffee grinder, Model 2044, to flours. Moisture and WRC were determined on these reconstituted flours.

#### WRC Test

Flour (5 g., 14% m.b.) was weighed into a tared 50-ml. centrifuge tube, 25 ml.

TABLE II. YIELDS AND PROTEIN CONTENTS OF FRACTIONS AND RECOVERIES FOR FOUR FLOUR-FRACTIONATION METHODS APPLIED TO A SWW FLOUR<sup>a</sup>

Fractionation Method	Fraction	Yield <sup>b</sup> %	Protein %	Recoveries	
				Dry matter %	Protein %
Standard: kneading of mech. mixed doughs	Water-sol.	4.5	14.9		
	Gluten	12.1	65.6		
	Tailings	24.3	1.0		
	Starch	58.2	0.3	99.1	98.7
Kneading: doughs made with salt sol.	Water-sol.	6.5 <sup>c</sup>	13.4		
	Gluten	11.5	67.1		
	Tailings	26.6	1.0		
	Starch	56.2	0.2	100.8 <sup>c</sup>	98.2
Kneading: doughs made by centrifuging	Water-sol.	5.4	17.5		
	Gluten	12.8	58.4		
	Tailings	28.2	1.7		
	Starch	52.8	0.3	99.2	99.3
Acid extraction	Water-sol.	5.0	17.6		
	Gluten	8.6	74.9		
	Tailings	17.8	3.6		
	Starch	64.7	0.3	96.1	89.4

<sup>a</sup>All these fractionations were with the Gaines soft winter wheat flour.

<sup>b</sup>All data are on a 14% m.b.

<sup>c</sup>These figures include some of the added sodium chloride.

distilled water was added, and the tube was stoppered. The tube was shaken vigorously and allowed to stand for 20 min., with shaking every 5 min. It was then centrifuged for 15 min. at 1,000 X g, the supernatant was decanted, and the tube was drained for 10 min. at a 45° angle. The tube was weighed, and the gain in weight was expressed as percent. Determinations were made in duplicate or more.

#### AWRC Test

This test was made exactly as described above, except that 0.1N sodium bicarbonate solution replaced the distilled water.

#### Analytical Methods

Conventional methods were used for moisture and protein (9). Particle-size distribution was determined according to AACC Method 50-10 (9). Both the 300- and 600-1,200-r.p.m. centrifuges were used. Damaged starch was estimated according to AACC Method 76-30 (9).

### RESULTS AND DISCUSSION

When the standard F&R method was applied in the WRC test, the resulting values were decidedly higher than expected (Table III). Several steps of the F&R method were then investigated. Ultimately one factor—reconstitution mixing time—proved to have some effect in lowering WRC of reconstituted flours. After

TABLE III. WRC OF FLOURS PREPARED BY THE STANDARD F&amp;R METHOD

	Normal Flours %	Reconstituted Flours %
HRW	68.2	78.9
SWW	52.3	58.7
Club	50.6	56.3

TABLE IV. EFFECT OF MIXING TIME ON WRC OF RECONSTITUTED FLOURS

	WRC						
	Normal flours %	Simple blends %	Reconstituted flours				
			Mixing time, min.				
			0.5 %	1.0 %	2.0 %	4.0 %	6.0 %
HRW	68.2	78.9	78.5	80.8	79.4	74.6	73.6
SWW	52.3	58.4	60.2	59.7	57.5	56.0	55.8

this was discovered, much of the experimental work was repeated, using the optimum reconstitution mixing time. Therefore the study of mixing time will be reported first.

#### Effect of Reconstitution Mixing Time on WRC

Normally, mixing during reconstitution has been continued until a dough of satisfactory appearance and feel was obtained. Usually this took 1 to 2 min. after formation of a dough from the dry fractions plus water. Table IV shows the effect of varying the mixing time. WRC values decreased as mixing time was increased up to 4 and 6 min.

#### WRC of Simple Blends of Fractions

A simple blend of fractions could be used directly in the bread-baking test in bread-baking studies (5,7). However, for cookie-baking (6,10), cake-baking (11), and MacMichael viscosity (4) studies, it was absolutely necessary to mix the fractions to a dough before the reconstituted product could be used in the baking or viscosity test. With water-retention tests, a simple blend of fractions gave a result slightly higher than that of reconstituted flours at optimum mixing time as shown in Table IV.

#### Effect on WRC of Omitting the Water-Soluble Fraction

Each of the three fractions—gluten, tailings, and starch—had a finite, characteristic WRC of its own (12). The water-soluble fraction had no apparent

TABLE V. EFFECT ON WRC OF OMITTING THE WATER-SOLUBLES FROM SIMPLE BLENDS AND RECONSTITUTED FLOURS

	Number of Fractions <sup>a</sup>	Sample Weight g.	Actual Gain in Weight g.	Gain if Weight of Water-Solubles Subtracted <sup>b</sup> g.
Simple blends				
HRW	3	4.74	4.23	...
	4	5.00	3.92	4.18
SWW	3	4.78	3.17	...
	4	5.00	2.91	3.13
Reconstituted flours				
HRW	3	4.74	3.77	...
	4	5.00	3.71	3.96
SWW	3	4.78	2.90	...
	4	5.00	2.77	2.99

<sup>a</sup>Three fraction samples had the gluten, tailings, and starch fractions in the proportions obtained in fractionation (adjusted to 14% m.b.). Four fraction samples had in addition the water-solubles.

<sup>b</sup>HRW water-solubles weighed 0.26 g. (5.2% yield), and SWW water-solubles weighed 0.22 g. (4.4% yield).

WRC as measured by the WRC and AWRC tests because this fraction dissolved completely in the solvent, and there was no centrifugate. In a dough, however, some constituents of this fraction do bind water. The addition of 1% water-soluble pentosans to bread doughs increased both the extensigraph absorption and the baking absorption (13). The water-soluble fraction had a large depressing effect on MacMichael viscosity of reconstituted flours (4). Therefore the effect on WRC of omitting this fraction was investigated.

With most tests this effect could be rather easily determined. In a cookie-baking study this fraction could be omitted, a flour could be reconstituted from the other three fractions and baked into cookies, and the diameter could be measured.

In the retention test, the result is calculated as a percent of the sample weight, and small differences in sample weight can cause large differences in the result. Omission of the water-solubles would give a smaller sample weight, and if this fraction were inert, would automatically cause a higher WRC. Therefore, a different way of expressing the results was adopted for this experiment to determine whether the water-solubles were contributing to retention or were merely an inert diluent (Table V).

The HRW three-fraction simple blend had an actual weight gain of 4.23 g. (line 1, Table V) and would have had a retention of 89 if a straightforward calculation had been made. In the HRW four-fraction blend, assuming that the water-solubles dissolved completely and were removed during centrifugation, and that the water retention of the four-fraction blend was due only to the gluten, tailings, and starch, then for comparison the deduction of the weight of water-solubles from the sample weight of the four-fraction blend would be justified. When this was done for the HRW blend, the gain in weight of the four-fraction blend (4.18 g.) was almost identical with that of the three-fraction blend. This was also true for the SWW simple blends (Table V).

The evidence was not so clear-cut for the reconstituted flours. With the HRW flour, the actual gain of the three-fraction reconstituted flour was only slightly more than that for the four-fraction flour. If the weight of the water-solubles were deducted, the four-fraction flour gained more than the three-fraction flour. The SWW three- and four-fraction flours also showed an intermediate pattern. It may be that, during doughing of the fractions in reconstitution, some binding of the water-solubles occurred, thus preventing solution of some of the water-solubles.

On the whole, however, the evidence supported the hypothesis that retention was due to the gluten, tailings, and starch, and that the water-solubles neither contributed to water retention nor caused the higher WRC of reconstituted flours.

#### Effect of Freeze-Drying the Fractions

A commonly used technique in F&R studies that would appear to be advantageous is freeze-drying of all flour fractions (5,6,7,11), although starch may be damaged by drying to very low moisture levels or require rehydration (7). Previous unpublished work here had shown that freeze-drying of all fractions did not improve the cookie- and cake-baking qualities of reconstituted flours. In the present study, two fractionations were made, in which the gluten, tailings, and starch, as soon as they were obtained in the wet form, were weighed and divided into halves. One-half of each fraction was then air-dried in the usual manner. The other half of the gluten was cut into small pieces, frozen, and freeze-dried. The tailings and starch were resuspended in water, shelled, and freeze-dried. Flours were reconstituted from either all air-dried or all freeze-dried fractions. The doughs for these flours were freeze-dried and ground by the standard reconstitution method. Table VI shows that for water retention both methods of drying the fractions gave the same results. It should be recognized, however, that for bread-baking freeze-dried gluten has been shown to be superior to air-dried gluten (14).

The standard reconstitution method has been to freeze-dry the doughs mixed from a blend of fractions and water. Air-drying of reconstituted doughs was investigated briefly by reconstituting doughs from blends of air-dried gluten, tailings, and starch, and freeze-dried water-solubles. The doughs were cut into thin strips, air-dried under room conditions, and ground to flours. Table VI shows that air-drying reconstituted doughs decidedly increased water retention.

TABLE VI. EFFECT ON WRC OF FREEZE-DRYING FRACTIONS COMPARED WITH AIR-DRYING AND OF AIR-DRYING RECONSTITUTED DOUGHS COMPARED WITH FREEZE-DRYING DOUGHS

	WRC	
	%	%
	HRW	SWW
Normal flours	67.8	52.2
Reconstituted flours		
Doughs freeze-dried		
From air-dried fractions	74.9	56.2
From freeze-dried fractions	74.6	57.1
Doughs air-dried		
From air-dried fractions	79.1	61.4

### Effect of Cold Fractionation

The standard method, except for temperature, was used to fractionate flours in a refrigerator room at 2°C. The flours, all other materials, and all equipment had been stored in the refrigerator room overnight, and crushed distilled water ice was added during kneading. WRC of the fractions, simple blends, and flours reconstituted from these fractions were determined. One run each was made for the HRW and SWW flours. As with the freeze-drying experiments, the results were within the range of values found for the standard method.

### Effect of Particle Size on Retention Capacities

In the standard reconstitution method, the freeze-dried doughs are ground on a coffee grinder at the next-to-finest setting. The effect of particle size on water retention was determined by reconstituting two 50-g. lots of the same HRW fractions and combining them after freeze-drying. The entire lot was ground at the coarsest possible setting, and a 25-g. aliquot was taken. The remainder was ground at the second-from-finest setting, and a 25-g. aliquot was taken. This was repeated

TABLE VII. EFFECT OF PARTICLE SIZE ON WRC OF RECONSTITUTED FLOURS

	Normal Flours	Reconstituted Flours			
		Grind			
		Coarse	Medium fine	Fine	Very fine
HRW					
Mass mean size, $\mu$	54	.. <sup>a</sup>	140 <sup>b</sup>	78	39
WRC, %	68.8	75.7	73.9	74.5	74.1
AWRC, %	70.2	77.1	75.5	77.3	80.0
SWW					
Mass mean size, $\mu$	45	.. <sup>a</sup>	134 <sup>b</sup>	73	34
WRC, %	52.6	57.5	56.8	56.1	57.1
AWRC, %	53.4	57.7	57.1	57.8	59.6

<sup>a</sup>This size was impossible to measure, as particles larger than 1 mm. bridged over the top of the bore.

<sup>b</sup>It was possible to measure this size, but rather large variations occurred because most of the material settled very rapidly.

TABLE VIII. MALTOSE AND DAMAGED-STARCH CONTENTS OF RECONSTITUTED FLOURS

		Maltose			Damaged Starch %
		Inherent mg.	Total mg.	Net mg.	
HRW	Normal flour	0.5	5.0	4.5	7.4
	Reconstituted	3.0	7.3	4.3	7.1
SWW	Normal flour	0.5	2.8	2.3	3.8
	Reconstituted	1.2	3.5	2.3	3.8

TABLE IX. WRC OF FLOURS RECONSTITUTED FROM THE FRACTIONS OF FOUR SELECTED FRACTIONATION METHODS

	WRC	
	%	%
	HRW	SWW
Normal flours as-is		
plus 1% sodium chloride	68.2	52.7
plus 2% sodium chloride	68.0	53.0
	67.4	52.9
Reconstituted flours from		
Kneading of mechanically mixed doughs	74.0	56.4
Kneading of mechanically mixed doughs; doughs formed with sodium chloride solution	71.8	48.1
Doughs formed by centrifugation; no mechanical mixing	76.5	56.7
Acetic acid extraction method	80.9	58.8

at the usual setting, and finally the remainder was ground at the finest possible setting. The same treatment was given to a 100-g. composite of two lots of SWW fractions from the same fractionation.

Table VII gives the mass mean size and retention capacities for these samples. Particle size had no effect on WRC, except that AWRC showed a small increase at the finest setting.

Gracza (15) has shown that, for air-classified fractions of normal flours, particle size has a large effect on AWRC and absorption. Freeze-dried reconstituted flours, however, have a porous structure. This may account for the fact that particle-size differences have little effect on water retention.

#### Damaged-Starch Content of Reconstituted Flours

Reconstituted flours had abnormally high inherent-maltose values (Table VIII). The total maltose values were also high, but if the conventional formula were used for calculation of damaged starch (9, Method 76-30) these two abnormally high values canceled one another, and the net maltose values and percent damaged starch for reconstituted flours were similar to those for the parent flours.

#### Effect of Fractionating Method

Three additional fractionation methods were studied. Flours were reconstituted from the fractions of each method, and retention values were determined and compared with those of the standard method. Reconstituted flours from the method avoiding mechanical mixing had either the same or higher WRC compared with flours from the standard method (Table IX). Mechanical mixing did not appear to be harmful in WRC studies. The use of sodium chloride solution to form the initial dough in kneading fractionations decreased WRC markedly. This was not due to the sodium chloride itself, as shown by the addition of sodium chloride to normal flours (Table IX), but must be caused by some protective action on the fractions during fractionation. Flours reconstituted from the fractions of the acetic acid separation had decidedly higher WRC. Thus only one fractionation method offered any possible improvement over the standard method. It was not adopted because the effects of sodium chloride solution on reconstituted flours were not fully understood.



### CONCLUSIONS

WRC of flours processed by the standard F&R method proved to be very stable although they did not match normal flour values exactly. Most of the variations in the F&R procedure that were investigated had very little effect on WRC. Only two factors, longer reconstitution mixing time and use of sodium chloride solution to form the dough at the beginning of fractionation, caused small improvements. Mechanical mixing during fractionation was harmless as far as water-retention tests were concerned. Freeze-drying of fractions, fractionation at zero temperatures, variations in particle size, and omission of water-solubles had no effect on water retention of reconstituted flours. Reconstitution by doughing of fractions was not absolutely necessary.

The higher inherent and total maltose contents of reconstituted flours were discovered. Although the damaged-starch content of reconstituted flours was apparently no higher than that of normal flours, the role of these constituents in reconstituted flours deserves further study.

Although the reconstituted flours had higher WRC than the parent normal flours, the increase was about the same for all varietal flours, and the difference due to variety remained. A F&R project to assess the relative contribution of each flour fraction to the varietal WRC difference appeared feasible, with due recognition that one or more fractions may have enhanced WRC because of flour fractionation.

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